Preliminary Amendment for Divisional Application of 10/110,945 Filed March 29, 2004 Page 2

Amendments to the Specification:

Page 1, before line 1 insert and add the following paragraph:

This application is a divisional of co-pending U.S. application serial number 10/110,945, filed April 14, 2002, which was a §371 national stage application of PCT/JP00/06820, filed on 2 October 2, 2000, the teachings of which are incorporated herein by reference in their entirety.

Please amend the paragraph beginning at page 30, line 4, as follows:

PCR reaction was performed in a 50 μl reaction mixture containing 40 mM Tricine-KOH, pH 9.2, 15 mM KOAc, 3.5 mM Mg(OAc)Mg(OAc)₂, 75 μg/ml bovine serum albumin, 200 μM dNTPs, 0.2 μM 270F or 92R, 0.2 μM adaptor primer 1 (Clontech), 5 μl of diluted adaptor-ligated double stranded cDNA solution, 1 μl of 50X Advantage cDNA Polymerase Mix (Clontech). PCR conditions were 94 °C 30s, followed by 5 cycles of 94 r 5s and 72 °C 2min, followed by 5 cycles of 94 °C 5s and 70°C 2min, and followed by 20 cycles of 94°C 5s and 68 °C 2min.

Please amend the paragraph beginning at page 31, line 8, as follows:

Rsa I-Rsa I cDNA fragment of mMincle (nucleotide 1188-1404), Sph I-Pst I Sph I-Sph I-CDNA fragment of mouse NF-IL6 (X62600 nucleotide 135-897), cDNA fragment of mouse MIT-2 (X53798 nucleotide 149-840), cDNA fragment of mouse glyceraldehyde-3-phosphate dehydrogenase (G3PDH; M32599 nucleotide 566-1017) were used as probes. cDNA fragments were radiolabeled with [α ³²P] dCTP (3000 Ci/mmole) by use of the Megaprime DNA labeling system (Amersham Pharmacia Biotech).

Please amend the paragraph beginning at page 31, line 17, as follows:

Interspecific backcross progeny were generated by mating (C57BL/6J x M. spretus) $\underline{F_1}$ females and C57BL/6J males as described (24). A total of 205 N_2 mice were used to map the Mincle locus.

Please amend the paragraph beginning at page 31, line 24, as follows:

The mMincle cDNA fragment (nucleotides 1549-2517) was labeled with $[\alpha^{-32}P]$ dCTP using a nick translation labeling kit; washing was done to a final stringency of 1.0 XSSCP, 0.1% SDS, 65°C. A fragment of $\frac{37 \text{ kb}}{3.7 \text{ kb}}$ was detected in BamHI digested C57BL/6J DNA and a fragment of 6.5 kb was detected in BamHI digested M. spretus DNA. The presence or absence of the 6.5 kb BamHI M. spretus-specific fragment was followed in backcross mice.

Please amend the paragraph beginning at page 32, line 24, as follows:

Amplified product was verified by sequencing after subcloned into pT7Blue T vector and excised by Sal I digestion. Then mMincleFlag cDNA fragment was ligated to Xho I site Xho site—of pcDNA3.1(+) with correct orientation. The day before transfection, 293T cells were seeded on 6-well plate at 2.0 X10⁵ cells/well. 4 μg of pcDNA3.1(+)-mMincleFlag or pcDNA3.1 empty vector was transiently transfected by calcium-phosphate precipitation method. Cells were freed from culture plates using 0.02 % EDTA in PBS at 48 h following transfection and washed in flow cytometry buffer (PBS with 2 % fetal bovine serum and 0.1 % NaN₃). Cells were incubated for 20 min on ice with 15 μg/ml biotin-conjugated anti-Flag M2 antibody (BioM2; Sigma-Aldrich, St. Louis, MO), washed in flow cytometry buffer, and labeled with 5 μg/ml FITC-streptavidin (Pharmingen, San Diego, CA). The cells were harvested 48 h after transfection for flow cytometric analysis. Control consisted of cells treated with FITC-streptavidin alone. After a final wash in flow cytometry buffer, mMincle-Flag expression was analyzed using FACS Calibur using CELLQuest software.

Please amend the paragraph beginning at page 34, line 13, as follows:

The 5.1kb Barn HI-Barn HI fragment containing exon I and 5'-flanking region was designated pBSBaml-8 and employed for promoter-luciferase construction.

Preliminary Amendment for Divisional Application of 10/110,945 Filed March 29, 2004 Page 4

Appropriate 5'-primers and common 3'-primer were synthesized to amplify the promoter regions of mMincle. The following primers were used to generate pGL3-1783/+69, pGL3-1190/+69pGL3-1190/-69, pGL3-240/+69, and pGL3-61/+69;

Please amend the paragraph beginning at page 34, line 22, as follows: -69_+69(5'-GAAGATCTCCCCTGGAAAGTGAGTCTTG-3').